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of

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for

SAMPLE COLLECTION SYSTEM FOR GEL ELECTROPHORESIS

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1 be used in either a horizontal or a vertical position. Typically the slab gels have a thickness
2 of about 0.5 mm to about 1.5 mm. The slab gels range from about 8 cm to about 50 cm high
3 and from about 10 cm to about 20 cm wide. Some commercially available slab gel systems
4 use pre-poured gel between disposable plates. Other systems use reusable plates into which
5 a liquid gel is poured and then solidifies through polymer cross-linking or other mechanisms.
6 In addition, pre-formed gels are also available.

7 Molecules such as proteins, polypeptides, and nucleic acids can be separated to form
8 distinct bands on a slab gel. The particles are separated to a high resolution, meaning that
9 particles with minute differences in charge and/or size are completely separated. A
10 researcher may use gel electrophoresis to determine characteristics of an unknown sample
11 such as size and charge of particles and the number of distinct fragments in the sample. Gel
12 electrophoresis is also used to separate and purify a particular desired protein or nucleic acid
13 from a mixture.

14 Gel electrophoresis is frequently used to determine the size of a DNA fragment.
15 Nucleic acid molecules such as DNA have a relatively constant charge to size ratio which
16 results in DNA molecules of the same size migrating uniformly in the gel. To determine the
17 size of the DNA molecule of interest, a DNA ladder is mixed with the sample of interest and
18 run on the gel. A DNA ladder consists of DNA fragments of known, but varying lengths.
19 For example a 1,500 base pair (bp) DNA ladder has DNA segments ranging from 100 bp to
20 1,500 bp in 100 bp increments. The position of the unknown DNA fragment on the gel
21 compared to the DNA ladder can be used to determine the size of the DNA fragment of
22 interest. Prior to running the samples of DNA fragments or proteins on the gel slab, the
23 samples are stained, radioactively labeled, or fluorescently labeled so that the position of the
24 sample bands on the gel can be determined.

The same technique can be used to separate a DNA fragment of interest from a mixture of DNA fragments. Frequently, a researcher is looking for a DNA fragment or other nucleic acid of an approximate length. This desired DNA fragment is obtained in a sample mixed with other DNA fragments. The sample is then mixed with a DNA ladder and separated by gel electrophoresis. The researcher can determine which band on the gel is the desired DNA fragment by the position of the DNA ladder and the bands from the DNA sample. Once the DNA fragment of interest is identified, the band must be removed from the gel for further analysis and use. However, for the separated particle to be used for further analysis and manipulation, the sample bands must be recovered from the gel. Traditionally, removing the bands from the gel has been difficult. The methods used can be labor intensive. Moreover, the sample of interest can be contaminated or otherwise damaged.

Some methods for removing a sample of interest from a gel involve mechanically excising the portion of the gel containing the sample of interest. The sample of interest is then removed from the excised portion of the gel by either chemical or heat extraction methods. The purity of the sample obtained from these methods depend in large part on the skill of the person excising the sample from the gel. The sample of interest may also be denatured by the heat or chemicals used to remove it from the gel. Moreover, the processes take a long time and are not easily automated.

Other methods of manually extracting the sample of interest from the gel have been developed. These methods use electrophoresis to drive the particles into a membrane with a high affinity for DNA placed immediately downstream from the band of interest. The band is then removed from the membrane. Placement of the membrane in the gel can cause a disruption in the electrical field causing the particles to migrate around the membrane which can result in a low collection efficiency or a contaminated sample band.

1 These and other manual methods of obtaining the sample of interest from a slab gel
2 have many defects. First, most of the methods are highly dependant on the skill of the
3 operator. The methods are also labor intensive which makes the process more time
4 consuming and costly. Moreover, the methods are not easily automated. Also, the methods
5 can result in the contamination of the sample of interest. The heat and chemicals used in
6 some of the methods may also damage the sample of interest.

7 Accordingly, a need exists for an apparatus that can automatically collect sample
8 bands from a gel electrophoresis system. It would be an additional advancement if the
9 apparatus, could cleanly extract the sample bands. It would be an additional advancement
10 if the accuracy of the device were not dependant on the skill of the operator. It would be a
11 further advancement if the system were capable of simultaneously extracting bands from
12 multiple sample lanes. It would be a further advancement if the system did not use chemicals
13 or temperatures which could damage the sample of interest. It would be an additional
14 advancement if the device could extract a sample band from a slab gel without over diluting
15 the sample. Such an apparatus is described and claimed herein.

16 17 **BRIEF SUMMARY OF THE INVENTION**

18 The apparatus of the present invention has been developed in response to the present
19 state of the art, and in particular, in response to the problems and needs in the art that have
20 not yet been fully solved by currently available systems and methods for collecting samples
21 from slab gels. Thus, it is an overall objective of the present invention to provide an
22 automatic collection system for collecting samples of interest from a gel electrophoresis slab
23 gel.

24 To achieve the foregoing objects, and in accordance with the invention as embodied
25 and broadly described herein in the preferred embodiments, an automatic sample collection
26

1 system for gel electrophoresis is provided. The system is adaptable to be used with a slab
2 gel with one or more lanes and may be retrofitted to existing slab gel systems. A sample of
3 particles such as DNA, RNA, polypeptides, and proteins can be separated into sample bands
4 on the slab gel. The sample collection system can automatically collect one or more of the
5 sample bands. The sample bands can then be further purified and used by an operator of the
6 sample collection system.

7 As the sample of particles is separated on the slab gel, the sample bands enter a
8 detection zone. A detector is positioned to detect the entry of a particle within the detection
9 zone. The detector can be used to scan all of the lanes of a multiple lane slab gel. Upon
10 detection of the particle, a syringe pump is energized. The syringe pump directs a stream of
11 buffer solution across a gel free zone within a lane of the slab gel. The buffer solution carries
12 the sample band from the gel free zone, through a collection port, and into a collection vial.
13 Generally the buffer solution used is the same buffer solution used in the slab gel.
14 Accordingly, the buffer solution may contain tris-boric acid EDTA hereinafter, TBE,
15 potassium tartrate, tris-acetate EDTA, hereinafter TAE, or other suitable buffers.

16 The detector of the present system can use a number of methods including UV-Vis
17 absorbance, fluorescence, raman, mass spectrometry; and electrochemical detection. In a
18 presently preferred embodiment, the detector uses fluorescence technology to detect the
19 sample bands. Generally a fluorescent tag is attached to the particles of the sample. A laser
20 can be positioned to excite the fluorescent tags on particles within the detection zone. The
21 laser is selected to have a wavelength that excites the fluorescent tag. Thus when certain
22 fluorescent dyes are used, an argon ion laser with a wavelength of about 488 nm is used.

23 An optical fiber can be imbedded in the gel to collect fluorescence from the excited
24 particles. The optical fiber is positioned adjacent the sample lanes and within the collection
25 zone. The fluorescence collected by the optical fiber is transmitted through the optical fiber
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The present invention also relates to a method of collecting a sample band from gel electrophoresis. The method comprises obtaining a sample of interest and fluorescently labeling the sample. The sample is loaded into a gel electrophoresis system with an automatic sample collection system of the present invention. The gel electrophoresis system is activated to separate the particles of the sample of interest into sample bands. A sample band is detected by the sample collection system. A syringe pump is then activated to direct a stream of buffer across a lane of the slab gel. The stream of buffer collects the sample bands and carries the band into a collection vial.

1 These and other objects, features, and advantages of the present invention will
2 become more fully apparent from the following description and appended claims, or may be
3 learned by the practice of the invention as set forth hereinafter.

4

5 **BRIEF DESCRIPTION OF THE DRAWINGS**

6 In order that the manner in which the above-recited and other advantages and objects
7 of the invention are obtained will be readily understood, a more particular description of the
8 invention briefly described above will be rendered by reference to specific embodiments
9 thereof which are illustrated in the appended drawings. Understanding that these drawings
10 depict only typical embodiments of the invention and are not therefore to be considered to
11 be limiting of its scope, the invention will be described and explained with additional
12 specificity and detail through the use of the accompanying drawings in which:

13 Figure 1 is a perspective view of one embodiment of an automatic collection system.

14 Figure 2 is an expanded cross sectional view of the embodiment of Figure 1 taken
15 across lines 2-2.

16 Figure 3 is an expanded cross section view of an alternative embodiment of the
17 invention.

18 Figure 4 is a graph illustrating laser induced fluorescence detection across multiple
19 lanes of a slab gel by a single laser.

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21 **DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS**

22 The presently preferred embodiments of the present invention will be best understood
23 by reference to the drawings, wherein like parts are designated by like numerals throughout.
24 It will be readily understood that the components of the present invention, as generally
25 described and illustrated in the figures herein, could be arranged and designed in a wide

1 variety of different configurations. Thus, the following more detailed description of the
2 embodiments of the apparatus, system, and method of the present invention, as represented
3 in Figures 1 through 4, is not intended to limit the scope of the invention, as claimed, but is
4 merely representative of presently preferred embodiments of the invention.

5 Referring now to Figure 1, an automatic collection system for use with a gel
6 electrophoresis system is generally designated 10. The system can be used with a standard
7 electrophoresis system 12. The automatic collection system 10 is depicted in conjunction
8 with a vertical electrophoresis system 12, however, the collection system 10 can also be used
9 with a horizontal electrophoresis system. The electrophoresis system 12 has a slab gel 14
10 positioned between two plates 16. The slab gel 14 is connected to an electrical source 18
11 which, when activated, applies a voltage across electrodes 21, 22. The voltage creates an
12 electrical field within the slab gel 14.

13 A mixture of particles to be separated and collected is inserted into wells 24
14 positioned near the top 20 of the slab gel 14. Particles such as nucleic acids, polypeptides,
15 and proteins are separated into distinct sample bands 26 as the particles migrate under the
16 influence of the electrical field. Generally an electrolyte buffer solution is contained within
17 the slab gel 14. The electrolyte buffer and gel allow a uniform electrical field to be
18 established within the slab gel and assures that the particles uniformly migrate based on size
19 and/or charge. Generally the electrolyte buffers used with standard electrophoresis systems
20 12 contain tris-boric acid EDTA hereinafter, TBE, and/or potassium tartrate, tris-acetate
21 EDTA, hereinafter TAE, but are not limited to these. The gels can also be polyacrylamide,
22 agarose, poly(ethylene oxide), and the like.

23 In certain configurations, the electrophoresis system 12 has two buffer reservoirs 94,
24 96 attached adjacent the top 20 and the bottom 23 of the slab gel 14. The buffer reservoirs
25 94, 96 can serve as the attachment point for the electrodes 21, 22. After the particles are
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1 The sample collection system 10 can be used to collect multiple bands from a single
2 lane 30. For example if a sample contains twenty different particles, a user may wish to
3 collect bands 2 and 7. The detector 32 will signal the computer 34 when band 1 passes, but
4 will not activate the syringe pump 36. Upon the detection of band 2, the syringe pump 36
5 will be activated and band two collected in a first sample vial 40. Bands 3 through 6 will
6 pass though the detection zone 28 without activation of the syringe pump 36. But when band
7 7 is detected, the computer 34 will activate the syringe pump again sending sample band 7
8 to a second collection vial 42. A fraction collector 44 can hold the multiple collection vials
9 38 and position a new vial 38 to be filled after each vial 38 is used.

10 The automatic sample collection system 10 can be configured to simultaneously scan
11 and collect samples from multiple lanes 30. A single detector 32 can be configured to scan
12 multiple lanes 30 for the presence of a sample band 26 within the lanes 30. When a desired
13 band 26 is detected within any of the lanes, the computer 34 signals one of an array of
14 syringe pumps 36 to activate. The computer 34 activates the syringe pump 36 corresponding
15 to the lane 30 wherein the sample band of interest is contained. The activated syringe pump
16 36 forces a stream of buffer across the lane 30. The sample band 26 of interest is collected
17 in a sample vial 38. An array of fraction collectors 44 can be used with a fraction collector
18 44 corresponding to each of the lanes 30 of the slab gel 14. Thus, the automatic collector 10
19 can simultaneously collect multiple sample bands 26 from multiple lanes 30.

20 The detector 32 can use a variety of detection methods such as raman and
21 radiolabeling to detect the presence of a sample band 26 within the detection zone 28. In the
22 illustrated embodiment, fluorescence technology is used for detection of the bands 26.
23 Fluorescent technology uses a fluorescent dye including, but not limited to PICO Green, TO-
24 PRO 1, and TO-PRO 3. The fluorescent dye is attached to the sample of interest. The
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1 fluorescent tags emit light when excited by a laser of a certain wavelength. The light from
2 the excited fluorescent tag is known as laser induced fluorescence.

3 The laser which is selected to excite the fluorescent tag depends on the tag itself. For
4 example, TO-PRO-3 intercalating dye (642/661 nm Ex/Em) can be easily excited with a
5 small HeNe laser at 635 nm. Using TO-PRO-3 allows the system to be quite sensitive,
6 however, the sensitivity of the system can be improved dramatically by using other dyes such
7 as PicoGreen (498/520 nm Ex/Em). PicoGreen can be excited with an argon ion laser at 488
8 nm. Using diode lasers at 500 nm can allow for fluorescent detection of the sample bands
9 26 while using a smaller laser.

10 In the illustrated embodiment, the sample of particles is labeled with a fluorescent
11 tag. A laser 50 directs a laser beam 52 into an optical fiber 54 coupled to the laser 50. The
12 optical fiber 54 transmits the laser beam 52 to the detection zone 28. A labeled band 26
13 within the detection zone 28 will fluoresce under the laser beam. The fluorescent light from
14 the band 26 is collected within a collection optical fiber 56 embedded in the gel 14. The
15 fluorescent light is transmitted by the optical fiber 56 to a light detector 58.

16 The light detector 58 can be low-level light detection electronics 60 such as
17 photomultipliers, photodiodes or CCD cameras. An optical filter 62 is positioned between
18 the fiberoptic 56 and the light detection electronics 60 to prevent incident light from the laser
19 beam 52 from reaching the detection electronics 60. The filter 62 is selected such that it
20 maximizes detection of the fluorescent light while minimizing or rejecting the background
21 light from the excitation beam hitting the detector. The optical filter 62 can be a
22 combination of a high band pass filter 62, for example greater than about 500 nm, with a
23 notch filter 62 at the wavelength of the laser beam 52 for example about 488 nm.
24 Alternatively, the filter 62 may be a narrow band pass filter 62 at the emission wave length
25 corresponding the fluorescence of the fluorescent tags. For example a narrow band pass
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1 filter 62 in the range of about 520 nm plus or minus about 10 nm has been used. Other
2 methods of filtering light such as a prism may be also used.

3 The laser beam 52 is focused on the detection zone 28 to achieve the maximum light
4 intensity without causing the fluorescent tags to photo-bleach. The focusing of the beam 52
5 also minimizes the width of the laser beam 52. Because the minimized beam 52 directs light
6 onto only a very small portion of the slab gel 16 at a given time, the system 10 can detect a
7 small separation distance thereby maximizing the detection efficiency of the system 10.

8 The power of the laser beam 52 can be optimized to provide maximum fluorescing
9 of the fluorescent tags while minimizing the background light transmitted to the light
10 detection electronics 60. A laser beam 52 with a power in the range from about 1 mW to
11 about 100 mW allows for sufficient fluorescing of the tags while minimizing the interfering
12 background. In a presently preferred embodiment of the system 10, the excitation beam has
13 a power of about 30 mW.

14 The optical fiber 54 is attached to a motorized X-Y stage 66 controlled by the
15 computer 34. The stage can move the fiberoptic head 64 of the optical fiber 54 to precisely
16 position the laser beam 52 in any of the detection zones 28. The motorized stage 66 can
17 move the position of the optical fiber head 64 back and forth across the slab gel 14 to direct
18 a single laser beam 52 on all of the lanes. In this manner the laser 50 can be used to detect
19 sample bands 26 in all lanes 30 of the system 10.

20 Referring now to Figure 2, a cross sectional view of a lane of the slab gel 14 is shown
21 within the detection zone 28. The slab gel 14 is bordered by a front plate 70 and a back plate
22 72. Each of the plates are fitted with a manifold 71, 73. The manifold 71, 73 allow access
23 through the plates 70, 72 to the slab gel 14. Generally the plates 70, 72 are made of glass.
24 However, when the manifolds 71, 73 are formed by drilling holes into the plates 70, 72, it
25 may be difficult to drill very small holes. In one embodiment the plates 70, 72 are made of
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1 polycarbonate. The polycarbonate plates 70, 72 are mechanically or chemically roughened
2 to prevent the gel 14 from migrating with the electric field. Generally the manifolds 71, 73
3 have an inside diameter in the range from about 1/64 inch to about 1/8 inch.

4 At the time of casting of the gel 14 between the plates 70, 72 a solid rod plug is
5 inserted into manifolds 71, 73. The plug is removed once the gel solidifies, leaving a gel free
6 zone 92. This zone 92 allows electromigration of analyte bands 26 while creating a low
7 pressure region for buffer liquid to flow from syringe 36, through the zone 92. The flowing
8 buffer collects an analyte bands 26, into the tube 84, and vial 38. Once the plug is removed,
9 an electrolyte buffer solution is injected into the gel free zone to allow the electrical field to
10 remain undisturbed within the gel free zone 92. As a practical matter the gel free zone 92
11 is aligned with the detection zone 28 allowing the bands 26 to be detected and collected,
12 generally simultaneously.

13 In a first manifold 71 a collection optical fiber 56 collects fluorescent light as the
14 laser beam 52 excites the sample band 26. The collection optical fiber 52 transmits the light
15 to the light detection electronics 60 which then signals the computer to activate the syringe
16 pump. The activated syringe pump 36 then sends a stream of buffer though a first capillary
17 78 though the gel free zone 92 and into a collection port 82 within the second manifold 73.
18 The stream of buffer 76 carries the sample band from the gel free zone 92 into the collection
19 port 82. From the collection port 82, the stream of buffer travels though a second capillary
20 84 into a sample collection vial 38.

21 Referring now to Figure 3, an alternative embodiment of automatic collection system
22 of the present invention is generally designated 110. The collection system 110 is shown
23 with a cross section taken through one of the detection zones 128. The system 110 uses the
24 same general components and parameters as the system 10. However, the laser beam 152
25 is not rastered between the detection zones 128 in the system 110. In the embodiments of
26

1 Figure 3, a fiber optic 154 is coupled to the laser 150. The fiber optic 154 transmits the laser
2 light 152, to a beam splitter 188 which divides the laser beam 152 into multiple laser beams
3 152. The beam splitter 188 transmits the split beams 152 to a series of transmission optical
4 fibers 180. Each of the multiple transmission optical fibers 180 directs the laser beam 152
5 into a detection zone 128 of a separate lane 130 of the slab gel 114.

6 The system 110 has a first manifold 171 and a second manifold 173. The
7 transmission optical fiber 180 enters the detection zone 128 through the second manifold
8 173. The laser beam 152 exits the transmission optical fiber 180 and excites a sample band
9 126 within the detection zone. A light collection optical fiber 156 enters the detection zone
10 128 through the first manifold 171 and collects fluorescent light as the laser beam 152
11 excites the sample band 26. The collection optical fiber 156 transmits the light to an optical
12 fiber switcher 186. From the optical fiber switcher 186, the fluorescent light is transmitted
13 through another optical fiber 190 to the light detection electronics 160 which then signal the
14 computer 134 to activate the syringe pump 136. The activated syringe pump 136 then sends
15 a stream of buffer through a first capillary 178 through the gel free zone 192 and into a
16 collection port 182. The stream of buffer 176 carries the sample band from the gel free zone
17 192 into the collection port 182. From the collection port 182, the stream of buffer travels
18 through a second capillary 184 into a sample collection vial 138.

19 When multiple lanes 130 are being monitored, multiple transmission optical fibers
20 156 can transmit fluorescent light to the optical fiber switcher 186 at the same time. The
21 computer 134 uses the switcher 186 to change between the optical fibers 156 so that light
22 from only one lane 116 at a time is sent to the light detection electronics 160. In this manner
23 the computer 134 distinguishes between the signal from the multiple lanes 130 and activates
24 the syringe pump 136 corresponding to the correct lane 130.

EXAMPLES

The following examples are given to illustrate various embodiments which have been made with the present invention. It is to be understood that the following examples are not comprehensive or exhaustive of the many types of embodiments which can be prepared in accordance with the present invention.

Example 1 - Fluorescence Detection across Multiple Lanes

An electrophoresis slab gel was used to separate a DNA sample. The gel is acrylamide buffer gel with TBE. The DNA sample was labeled with a fluorescent dye. About 3 kV was applied to the slab gel for about 24 hours. A 488 nm laser with an optical fiber head was scanned across the eight lanes of the gel using a motorized X-Y table. Fluorescence was detected by a reflectance probe positioned within the gel. The reflectance probe had an optical fiber head which collected any light from the fluorescing particles and transmitted the light to a photomultiplier tube. As shown in the electropherogram of Figure 4, the fluorescently labeled DNA sample bands were detected in all lanes of the slab gel.

SUMMARY

In summary, an automatic sample collection system for use with an electrophoresis system is presented. A detector is provided to detect the presence of a sample band within a detection zone. When a desired sample band is detected a syringe pump containing a buffer solution is energized and directs a buffer solution across a lane of a slab gel. The buffer solution can be the same buffer solution as that used in the slab gel. The buffer solution collects the sample band and transports it to a collection vial. The system can use fluorescence detection to detect the particle bands. A system is

1 provided capable of collecting multiple sample bands from multiple lanes of the slab gel.
2 The entire collection process can be automated and controlled by a computer.

3 The present invention may be embodied in other specific forms without departing
4 from its structures, methods, or other essential characteristics as broadly described herein
5 and claimed hereinafter. The described embodiments are to be considered in all respects
6 only as illustrative, and not restrictive. The scope of the invention is, therefore, indicated
7 by the appended claims, rather than by the foregoing description. All changes that come
8 within the meaning and range of equivalency of the claims are to be embraced within
9 their scope.

10 What is claimed and desired to be secured by United States Letters Patent is:
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